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EFFECT OF WETTABLE POWDER FORMULATIONS ON GERMINATION OF *NOMURAEA RILEYI* CONIDIA AT DIFFERENT STORAGE INTERVALS

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ABSTRACT

Wettable powder formulations of N. rileyi were were prepared at S.V. Agricultural College, Tirupati during 2013-14 and evaluated for testing the viability under two different storage conditions i.e., refrigeration(at 4ºC) and incubation (at 25 ºC) at monthly intervals upto 3 months.Among the storage conditions, refrigerator stored formulations shown better results when compared to incubator stored formulations. Among the different formulations, talc formulation shown higher viabilities upto 90 days of storage. Where as least germination was recorded with ragi flour formulation. The viability of N. rileyi conidia is gradually decreasing with increase in storage period.

KEYWORDS: Nomuraea Rileyi, Wettable Powders, Viability

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Original Article

INTRODUCTION

Other alternatives to chemical pesticides include entomopathogenic fungi, bacteria, viruses, protozoans and nematodes. Among different entomopathogenic fungi, *Nomuraea rileyi* is a potential candidate for use as a microbial insecticide (Ignoffo, 1981). The entomopathogenic fungus, *N. rileyi* is unable to form epizootics under low relative humidity conditions with higher temperature. In combating this problem development of suitable formulation plays an important role. A good formulation helps in preserving organisms, delivering them to their target and to improve their activities. Formulation is nothing but a technical concentrate of an organism that has been formulated. In formulation, priority is the retention of viability and virulence of the infective units during storage and application. Biological and physical properties of the formulation must remain stable for at least one year, but preferably for more than 18 months for commercialization to take place (Couch and Ignoffo,1981). Little information is available on the effect of formulations on conidia of entomopathogenic fungi. Hence formulations were prepared to evaluate viability of conidia of *N. rileyi*.

MATERIALS AND METHODS

To evaluate the viability of *N. rileyi*, WP formulations were prepared by using six inert materials *i.e.,* Talc, starch, rice flour, jowar flour, wheat flour and ragi flour. Hundred gm of each carrier was taken in 250 ml conical flasks and placed in an oven at 160°C for 1 hour on each day for 2 days until for sterilization. After sterilization, the carriers were mixed each with 2.5 gm of harvested *N. rileyi* spores under aseptic conditions. Two to three drops of tween-20 was added to the mixture as wetting agent for uniform mixing of spores with carriers. Each material was separated into 2 halves. One half was stored in refrigerator at 4 ºC and another in incubator at

25ºC. The following are the formulations made by using spore mass (WP formulations) *i.e.,* Talc formulation, starch formulation, rice flour formulation, wheat flour i.e., formulation, jowar flour formulation and ragi flour formulation

0.5 gm of each carrier was weighed separately and mixed with 100 ml of sterile distilled water (after addindg 2-3 drops of Tween-20) in 250 ml beakers. Later this suspension was serially diluted for three times to get $1x10^5$ spores ml^{-1} concentration. (From the four different concentrations prepared from stock suspensions, $1x10^5$ concentration was used for conducting the viability studies with 30 days intervals upto 90 days for all the formulations(which were stored in refrigerator and incubator)

2-3 drops of spore suspension of $1x10^5$ spores ml^{-1} concentration was placed in cavity slide. The cavity slide was placed in the humidity chamber which was prepared by arranging moistened cotton in petriplates and it was incubated at 22°C. At 12 hours intervals, the spore suspension was observed under microscope for counting the germinated spores. The germination percentage of *N. rileyi* was calculated based on the number of spores germinated in spore suspension of each formulation.

RESULTS AND DISCUSSIONS

Viability of Wettable Powder Formulations

At Refrigerated Conditions (4°C)

On the Day of Storage (ODS): The mean conidial germination was 87.22 per cent.

Thirty days after storage (30 DAS): Talc and rice flour formulations are statistically on par with each other with a germination per cent of 87. Wheat flour, jowar flour and ragi flour formulations recorded 80, 77 and 73 per cent of germination respectively. Where as Starch formulation has recorded highest (90) germination percentage.

Sixty Days After Storage (60 DAS): Higher conidial germination per cent of *N. rileyi* at the end of 60 days, was recorded in starch formulation (87 per cent) where as least conidial germination was recorded in ragi flour formulation (67 per cent). Mean conidial germination was reached to 77 per cent at the end of 60 days of storage.

Ninety Days After Storage (90 DAS): At the end of 90 days, mean conidial germination was reached to 70 per cent. Talc formulation was found superior by recording 80 per cent of conidial germination followed by starch 77 per cent. (Table.1)

At Incubated Conditions (22°C)

On the Day of Storage (ODS) : The mean conidial germination at the time of storage was 87 per cent.

Thirty Days After Storage (30DAS): Starch formulation recorded 90 per cent germination of *N. rileyi*, where as talc formulation recorded 87 per cent of germination. In rice flour formulation 83.33 per cent of *N. rileyi* conidia was recorded. Wheat flour, jowar flour and ragi flour recorded 77, 73 and 70 per cent of germination of *N. rileyi* conidia.

Sixty Days After Storage (60 DAS): At the end of 60 days, Talc formulation recorded highest germination percentage of 80, where as starch formulation was occupied second position with a germination per cent of 77.

After 60 days of storage, all the formulations maintained more than 60 per cent of germination of *N. rileyi*. The mean per cent conidial germination after 60 days of storage was 71.67 per cent.

Ninety Days After Storage (90 DAS): Among all the formulations, talc flour was found superior by recording 77 per cent of conidial germination of *N. rileyi*. Except talc, in all other formulations less than 70 per cent of germination of *N. rileyi* conidia was observed. (Table 2).

With regarding to viabilities of incubator stored and refrigerator stored wettable powder formulations, higher germination percentages were recorded in refrigerator stored formulations. This may be due to the fact that under refrigerated conditions, the life activity will be at its minimum. So there may be a negligible metabolic activities taking place within the living organism. This may be the reason for less loss in viability of *N. rileyi* conidia under refrigerated conditions.From these studies it can be concluded that *N. rileyi* formulations can be stored safely for more than 3 months both in refrigerator and incubator (because only around 3 per cent difference with loss of viability was observed in all the formulations) conditions.

The present results indicated that the viability of *N. rileyi* conidia reduces with increase in storage period. Refrigerator stored talc formulation showed less reduction of per cent of conidial germination of 10 per cent, where as talc formulation stored at 25°C recorded 13.33 per cent reduction of conidial germination from the day of storage to 90 days after storage. Chaudary *et al.,* 2001 reported that talc formulations of *Beauveria bassiana* recorded 13% reduced viability after the storage for 90 days.

In the present study, talc was found to be the best carrier material for maintenance of viability of *N. rileyi* conidia because about 76 per cent of germination was observed even after 3 months of storage in both storage conditions). The results are similar with the results of Swetha (2011) who prepared wettable powders of *N. rileyi* with various inert materials and evaluated the efficacy and viability against *S. litura*. She confirmed that talc formulation was most efficient material followed by starch and rice flour for maintaining the viability of *N. rileyi* conidia (above 30 per cent) even after 240 days of storage. Srikanth *et al.*(2006) who prepared dry formulations of *B. brongniartii* with various inert material and evaluated for conidial viability and virulence against *Holotrichia serrata* and concluded talc, lignite materials were recorded long shelf life.

In the present study, upto 30 days of storage, starch formulation was occupied first place by recording 90 per cent viability, but at the end of 60 days of storage talc occupied the first place with a conidial germination of 80per cent at the end of 60 days of storage. Das *et al*. (2006) assessed growth and sporulation of *Trichoderma harzianum* in formulations made with different carriers, starch, talc and molasses in nine combinations. Among nine formulations tested, starch showed maximum sporulation upto 60 days but from 60 days onwards talc based formulation gave higher sporulation than starch.

In present studies, talc formulation have shown promising results in maintaining conidial viability of *N. rileyi* in both storage conditions *i.e.,* at 4°C (80%) and 25 °C (77%). Among refrigerator stored formulations, talc and starch emerged as the best, where as Jowar flour and ragi flour formulation appeared to be least effective formulations. The same evident was also recorded with incubator stored starch, talc and jowar flour, ragi flour formulations. On the other hand, ragi flour formulation showed the least germination percentage in both storage conditions. This may be due to less nutrient status of ragi grain compared to other inert materials.

The present results indicated that the viability of *N. rileyi* conidia reduces with increase in storage period. This findings were in close association with the following scientists. Ramegowda (2005) evaluated the viability of *N. rileyi*

conidia in the wettable powder formulations containing different carrier materials and storage environments. His experimental results revealed that there is depletion in viability of conidia with increase in storage period and temperature. He mentioned that viability of spore was significantly influenced by the duration of storage. And also reported 82.47 per cent conidial viability of *N. rileyi* formulations stored for 180 days in refrigerated condition compared to 63.23 per cent under room temperature formulation with talc. Rachappa (2003) reported 50.28 per cent conidial germination in *M. anisopliae* wettable powder formulation stored for 180 days in ambient room temperature compared to 70.4 per cent under refrigerated conditions.

CONCLUSIONS

More than 80 per cent of conidial germination of *N. rileyi* was recorded in the findings of Wiwat(2004) even after 23 weeks of storage at 4°C of formulations involving bentonite and its combination with sugars at the ratio of 7:1. Similarly when Daust *et al.* (1983) formualated the *M. anisopliae* conidia with dust formulation in kaolinite, they recorded higher viabilities(80%), when compared to un formulated one.

REFERENCES

1. *Chaudary, D. K. R.., Kanaujia & Rathore, R. R. S. 2001. Mycopesticidal dust formulations: Evaluation of diluants for viability of* Beauveria bassiana *(Balsamo) vuillemin.* In: Proceedings of symposium on biocontrol based pest management for quality crop protection in the current millennium. *87: 18-19.*
2. *Das, B.C., Das, B.K., Pranab, D & Smah, K. 2006. Bio formulation of* Trichoderma harzianum *for management of soybean stem rot caused by* Rhizoctonia solani *Kuhn.* Journal of Biological control. *20(1):57-64.*
3. *Daust, A. R., Ward, G. M. & Roberts, W. D. 1983. Effect of formulation on the viability of* Metarhizium anisopliae *conidia.* Journal of Invertebrate Pathology. 41 *: 151-160.*
4. *Ignoffo, C. M. 1981. The fungus* Nomuraea rileyi *as a microbial insecticide. In :* Microbial Control of Pests and Plant Diseases. *1970-80. BURGES, H. D. Ed., Academic Press, New York, pp. 513-536.*
5. *Rachappa, V. 2003. Occurrence of entomopathogenic fungi and utilization of* Metarhizium anisopliae *(Metschnikoff) Sorokin in the management of selected crop pests in north Karnataka.* Ph. D. Thesis, *University of Agricultural Sciences, Dharwad.*
6. *Ramegowda, G.K. 2005. Aerobiology, Epizootiology and Utilization of Nomuraea rileyi (Farlow) Samson. Ph. D. Thesis, University of Agricultural sciences, Dharwad, Karnataka.*
7. *Srikanth, J., Santhalakshmi, G & Tamizharasi, V. 2006.Viability and Virulence of selected* Beauveria brongniartii *formulations against* Holotrichia serrate. Sugar Technology. *8(2 & 3): 152-154.*
8. *Swetha, K. 2011. Evaluation of dry formulations of Nomuraea rileyi (Farlow) Samson and molecular characterization of its isolates. M.S.c.(Ag.) Thesis. Acharya N.G. Ranga Agricultural University.*
9. *Wiwat, C. 2004. Development of* Nomuraea rileyi *based biopesticide for controlling lepidopteran larvae.* Ph. D. Thesis, *Mahidol University, Malaysia.*

APPENDICES

Table 1: Viability of *N.rileyi* in Terms of Germination of Conidia in Refrigerator Stored WP Formulations

Formulations	Percent Germination of N.Rileyi Spores(DAS-Days After Storage, ODS-on the Day of Storage			
	ODS	30DAS	60DAS	90DAS
Talc	90.00 [b] (71.57)	86.67 [b] (68.59)	83.33 [ab] (65.92)	80.00 [a] (63.45)
Starch	93.33 [a] (75.15)	90.00 [a] (71.62)	86.67 [a] (68.60)	76.67 [a] (61.13)
Rice flour	90.00 [b] (71.58)	86.67 [b] (68.60)	80.00 [b] (63.45)	73.33 [b] (58.92)
Wheat flour	86.67 [c] (68.60)	80.00 [c] (63.45)	73.33 [c] (58.92)	66.67 [c] (54.77)
Jowar flour	83.33 [d] (65.92)	76.67 [d] (61.12)	70.00 [cd] (56.80)	63.33 [cd] (54.77)
Ragi flour	80.00 [e] (63.44)	73.33 [e] (58.92)	66.67 [d] (54.75)	60.00 [d] (50.77)
General mean	87.22	82.22	76.67	70.00
SE(m)	0.75	0.72	0.79	0.93
C.D.(0.05)	2.10	2.02	2.21	2.61

- The values are means of three replications.
- Figures in the parentheses are angular transformed values.
- Mean followed by same letter in the column do not differ significantly by

  DMRT (p = 0. 0 1)

Table 2: Viability of *N.Rileyi* in Terms of Germination of Conidia in Incubator Stored WP Formulations

Formulations	Percent Germination of *N.Rileyi* Spores (DAS-Days After Storage, ODS- On the Day of Storage)			
	ODS	30DAS	60DAS	90DAS
Talc	90.00 [b] (71.62)	86.67 [a] (68.60)	80.00 [a] (63.45)	76.67 [a] (61.14)
Starch	93.33 [a] (75.15)	90.00 [a] (71.62)	76.67 [ab] (61.15)	70.00 [b] (56.79)
Rice flour	90.00 [b] (71.58)	83.33 [a] (65.92)	73.33 [bc] (58.92)	66.67 [c] (54.75)
Wheat flour	86.67 [c] (68.60)	76.67 [c] (61.15	70.00 [cd] (56.79)	56.67 [d] (48.83)
Jowar flour	83.33 [d] (65.91)	73.33 [cd] (58.93)	66.67 [de] (54.75)	53.33 [e] (46.91)
Ragi flour	80.00 [e] (63.43)	70.00 [b] (56.79)	63.33 [e] (52.73)	50.00 [f] (45.00)
General mean	87.22	80.63	71.67	53.53
SE(m)	0.71	0.77	0.80	0.69
C.D.(0.05)	1.98	2.16	2.22	1.92

- The values are means of three replications.
- Figures in the parentheses are angular transformed values.
- Mean followed by same letter in the column do not differ significantly by

  DMRT (p = 0. 0 1)